

AMENDMENTS TO THE SPECIFICATION

Please direct entry of the attached "Sequence Listing" in the above-identified patent application. The contents of the paper and the computer readable form of the Sequence Listing submitted herewith are the same and include no new matter, as required by 37 C.F.R. §§ 1.821(e)-(g), 1.825(b), and 1.825(d).

Appendix: Copy of the "Sequence Listing"

Please replace the paragraph at page 59, lines 2-8, with the following amended paragraph.

5 pmol of a DNA oligonucleotide containing the NF- κ B consensus binding site (5'-AGTTGAGGGGACTTTCCCAGGC-3') (SEQ ID NO:1), 5 μ l 5X forward reaction buffer, 10 units T4 polynucleotide kinase, 2.5 μ l [32 P]ATP (10 μ Ci/ μ l, 300 Ci/mmol), and water (to 25 μ l total volume) were incubated for 10 minutes at 37°C. The reaction was stopped by heating the mixture for 10 min at 65°C and the labeled oligonucleotide was separated from unincorporated [32 P]ATP by centrifuging the mixtures at 12,000-16,000 g for 30 seconds in G-25 Sepharose columns. The labeled nucleic acid was recovered in the collection tube in approximately 25 μ l of TE buffer.

Please replace the paragraph at page 59, line 18, to page 60, line 11, with the following amended paragraph.

Activation of NF- κ B was also determined using the Trans-AM™ assay (Active Motif, Carlsbad, California) per the manufacturer's instructions. (Renard et al., 2001) Briefly, cell monolayers on 60mm dishes were washed with ice-cold PBS and removed by incubating in trypsin-PBS or scraping. The cells were centrifuged for 10 minutes at 1,000 rpm at 4°C and resuspended for 10 minutes in 100 μ l of 4°C lysis buffer (20 mM HEPES (pH 7.5), 350 mM NaCl, 20% glycerol, 1% Igepal-CA630, 1 mM MgCl₂, 0.5mM EDTA, 0.1mM EGTA, 1 μ l of 1 M dithiothreitol and 10 μ l of protease inhibitor cocktail (proprietary) per ml of lysis buffer). The lysates were centrifuged for 20 minutes at 14,000 g at 4°C. Lysates containing 5 μ g of total protein were added to 20 μ l of lysis buffer per well in 96-well dishes containing immobilized oligonucleotides corresponding to the NF- κ B consensus DNA binding site (5'-GGGACTTTCC-

3') (SEQ ID NO:2). The plate was covered with an adhesive film and incubated for 1 hour at room temperature on a rocker. The wells were then washed three times with 200 µl per well of washing buffer (100 mM phosphate buffer (pH 7.5), 500 mM NaCl, 1% Tween). 100µl of p65 subunit monoclonal antibody (the p65 antibody supplied with the kit only recognizes p65-containing NF-κB heterodimers that are bound to DNA containing the NF-κB consensus binding sequence) was diluted 1:1,000 in 1X antibody binding buffer (4mM HEPES (pH 7.5), 120mM KCl, 8% glycerol, 1% bovine serum albumin) and added to each well and incubated for 1 hour at room temperature. The wells were washed with 100µl of washing buffer three times. 100µl of horseradish peroxidase-conjugated secondary antibody diluted 1:1,000 in 1X antibody binding buffer was added to each well and incubated for 1 hour at room temperature without agitation. The wells were washed four times with 200µl per well of 1X washing buffer. 100µl of developing solution (tetramethylbenzidine in 1% DMSO) was added to each well and incubated for 10 minutes at room temperature. 100 µl of stop solution (0.5 M H₂SO₄) was added to each well after which the absorbance was determined on a plate spectrophotometer at 450nm. Specificity of binding was determined using 200-fold excess wildtype NF-κB oligonucleotides added at the time the cell lysates were added to the wells.